

METHOD FOR SAMPLE INJECTION IN MICROCHANNEL DEVICE

BACKGROUND

This invention relates to microfluidic manipulations in microchannel structures.

Considerable attention has been directed to developing microchannel structures having capillary dimensions, in which small volumes of liquids and small quantities of materials carried in liquids can be transported electrokinetically, that is, under the driving force of an applied electric field. Application of an electric field to a liquid (such as a solvent) in a microchannel results both in a bulk flow of the liquid and of materials carried in it (such as solutes) owing to electroosmotic movement of the liquid, and in electromigration of the materials themselves in the liquid. Accordingly, electromigration can be used to separate materials that have different electrophoretic mobilities in the liquid, and both electroosmotic flow and electromigration can be used to transport substances from point to point within the microchannel device.

A variety of approaches have been described for employing electroosmotic flow to carry out valveless injections of samples in microchannel devices.

D. J. Harrison et al (1992), "Capillary Electrophoresis and Sample Injection Systems Integrated on a Planar Glass Chip", *Anal. Chem.* 64:1926-32, proposes a scheme for valveless switching of fluid flow in channels intersecting at a T-junction. In this scheme, a sample supply channel, a "mobile phase" supply channel, and a separation channel meet at a common intersection point. An electrode is placed at an inlet at the head of each channel. A sample containing a mixture of fluorescent dyes is introduced by syringe into the sample supply channel, and then the mobile phase supply channel and the separation channel are flushed by syringe with buffer. Then a voltage is applied between reservoirs at the heads of the sample supply channel and the separation channel, causing the sample solution in the sample supply channel to move into and along the separation channel past a fluorescent detector. According to this scheme a plug of sample can be injected into the separation channel from the sample supply channel by applying the voltage across the sample supply reservoir and separation reservoir for a brief period, and then allowing the potential at the sample supply reservoir to "float" (that is, disconnecting it from both ground and power supply) while applying a voltage across the mobile phase supply and separation reservoirs to move the plug in the separation channel and effect the separation. In practice, some sample material may leak from the sample supply channel by diffusion or convection at the intersection point during the separation phase. This leakage can be reduced by applying a voltage across the mobile phase supply reservoir and the sample supply reservoir after the injection phase, drawing solvent back into the sample supply channel and displacing the sample away from the intersection.

A different scheme is described in S. C. Jacobsen et al. (1994a), "Effects of Injection Schemes and Column Geometry on the Performance of Microchip Electrophoresis Devices", *Anal. Chem.* 66:1107-13. In this scheme, four channels meet at a common intersection, forming an "injection cross". Thus, an analyte supply channel runs from an analyte reservoir to the injection cross, an analyte waste channel runs from the injection cross to an analyte waste reservoir, a buffer supply channel runs from a buffer reservoir to the injection cross, and a separation channel runs

from the injection cross to a waste reservoir. The device is operated in a "sample loading mode" and a "separation mode". Two types of sample introduction are described for the sample loading mode. In a "floating" type of sample loading, a voltage is applied to the analyte reservoir with the analyte waste reservoir grounded, and with the buffer and waste reservoirs floating. As sample is drawn from the sample reservoir through the injection cross and into the sample waste channel, some sample moves laterally into the buffer supply channel and the waste channel. In a "pinched" type of sample loading, a voltage is applied to the analyte, buffer and waste reservoirs with the analyte waste reservoir grounded. As sample is drawn through the intersection the sample stream is constrained by streams of buffer entering from the buffer and waste reservoirs. After either pinched or floating sample loading, the device is switched to the separation mode. Here, a voltage is applied to the buffer reservoir with the waste reservoir grounded. To achieve a clean break of the injection plug, which is said to be mandatory to avoid tailing, buffer is drawn from the buffer channel into the analyte, analyte waste, and separator channels simultaneously, by holding the voltage at the intersection below the potential of the buffer reservoir and above the potential of the other three reservoirs, displacing the sample in the sample supply and sample waste channels away from the intersection.

A "gated valve" injection scheme employing an injection cross is described in S. C. Jacobsen et al. (1994b), "Microchip Capillary Electrophoresis with an Integrated Postcolumn Reactor", *Anal. Chem.* 66:3472-76. Here, a voltage is continuously applied to the analyte reservoir with the analyte waste reservoir grounded, so that sample is continuously drawn from the analyte reservoir to the intersection and then laterally from the intersection to the analyte waste reservoir. Simultaneously a voltage is applied to the buffer reservoir with the waste reservoir grounded, to deflect the analyte stream and prevent the sample from migrating into the separation channel. To allow the sample to migrate from the analyte supply channel across the intersection into the separation channel, the potentials at the buffer and analyte waste reservoirs are floated for a short period of time. To separate a plug of sample as it passes into the separation channel, the voltage at the buffer and analyte waste reservoir are reapplied.

Similar schemes, and variations on them, are described in International Patent Publication WO 96/04547.

Published European Patent Application EP 0 620 432 describes an "offset T" microchannel configuration for sample loading. Here supply and drain channels open by way of respective supply and drain ports into an electrolyte channel. The distance between the supply and drain ports along the electrolyte channel defines a fixed sample volume. The sample is loaded by applying a voltage across the supply and drain channels for a time at least long enough that the sample component having the lowest electrophoretic mobility is contained within the geometrically defined sample volume. Then a voltage is applied along the electrolyte channel to move the sample plug and separate the sample. Preferably, after sample loading and prior to separation, electrolyte buffer is allowed to advance into the supply channel and the drain channel from the electrolyte channel, pushing sample back into those channels and away from the sample plug in the electrolyte channel.

SUMMARY OF THE INVENTION

In one general aspect, the invention features a method for transporting a liquid sample into a third microchannel from